Bacterial Immunofluorescence protocol

(Adapted from Jose et al., 2005, doi: 10.1016/j.ab.2005.08.019)

1) For every cell type that needs testing, grow a culture of bacterial cells in 5mL LB (+antibiotics) overnight at 37 ˚C.
2) Next morning, take OD_{600} of the cultures (OD of 1 for *E. coli* corresponds to ~10^8 cells/mL), and dilute into 2 fresh 5mL LB tubes (+antibiotics) to OD_{600} of ~0.01. To one of these tubes, add IPTG to end concentration of 1mM. Incubate both tubes in a 37 ˚C shaker.
3) After ~3 hr of incubation, start monitoring OD of the cultures every half hour. We want to fix these cells at an OD_{600} of ~0.5.
4) As soon as a culture reaches OD_{600} of ~0.4-0.5, spin down 1mL of the culture in an Eppendorf tube at 8000xg (=rcf) for 1 min, and carefully discard the supernatant (be careful so as to only remove the supernatant, without disturbing the cells in the pellet). (In the end I spun down 2mL of each and then combined the pellets (this worked much better, so I'd recommend doing that)).
5) Re-suspend the pellet in 1mL 1xPBS by pipetting up and down 5 times. Spin down the cells at 8000xg (=rcf) for 1 min, and carefully discard the supernatant.
6) Repeat the PBS wash in Step-5 two more times, but this time only use 0.5mL PBS.
7) Now, re-suspend the cells in 0.5mL 1xPBS by pipetting.
8) Mix 500 uL Blocking buffer with the annealed oligo (5.13uL) for each cell type in a separate Eppendorf tube, then add this to your cells.
9) Spin down the cells at 8000xg (=rcf) for 1 min, and carefully discard the supernatant.
10) Do 1x PBS wash (0.5mL PBS).
11) Now, fix the cells (in the tube itself) by resuspending in 1xPBS+4%(para)formaldehyde (500uL). Incubate at room temperature for 20 min.
12) Do 1x PBS washes (0.5mL PBS)
13) Drop 50uL of the resuspension on a coverslip (round coverslips preferred), and incubate at 37 ˚C until it is completely dry. Once dry, save the coverslip at RT until all the cultures have been processed similarly.
14) Add a drop of the mounting medium (ProLong Diamond Antifade Reagent, Fisher #15372192) on a glass slide and place the coverslip on top of it (bacterial-side-down).
15) Seal the edges of the cover-slip with nail-polish, and save in the fridge (4˚C), for later visualization.